by the current amendments. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

I. AMENDMENTS

In the Claims:

Please amend claim 34 as follows:

34. (AMENDED) Isolated nucleic acid encoding a humanized variant of a parent anti-VEGF antibody which parent antibody comprises non-human variable domains, wherein said humanized variant:

(a) binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said K_d value being no more than about 6-fold of the K_d value of said parent antibody;

(b) has an ED50 value of no more than about 5nM for inhibiting VEGF-induced proliferation of endothelial cells *in vitro*; and

(c) inhibits VEGF-induced angiogenesis *in vivo*, wherein 5mg/kg of said humanized variant inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model.

II. REMARKS

A. Regarding Claim Amendments

Claims 34-38 are currently under consideration. Claim 34 is hereby amended. Support for the amendments can be found in the specification as originally filed, for example, at page 14, line 30 to page 15, line 1 (for "parent antibody"); page 13, lines 2-3, page 23, lines 27-29 and Example 1 at page 50, lines 1-3 (for "non-human variable domains"); and page 67, line 23 (for "no more than about 6-fold"). Therefore, the amendments do not introduce new subject matter.

B. Sequence Listing

An Advisory Action was issued in response to the Sequence Listing submitted May 15, 2001. The Advisory Action stated that the submission was not entered for failing to state that no new matter is added. Applicants hereby resubmit the Sequence

Listing with a Certificate stating that the submission does not include new matter. Entry of the Sequence Listing is respectfully requested.

C. Related Applications

Applicants note that the present application is related to a co-pending application Serial No. 09/056,160, filed April 6, 1998. Applicants further note that another related application Serial No. 09/056,161 (filed April 6, 1998) has been expressly abandoned by the Applicants as of August 31, 2001.

D. Rejection under 35 U.S.C. §103

Claims 34-38 remain rejected under 35 U.S.C. §103 as allegedly being unpatentable over WO 94/10202. The claims are said to be rendered obvious because it was well within the level of the skill in the art to use conventional methods to produce a humanized murine anti-VEGF antibody with a binding affinity of 1x 10^{-9}, as suggested by WO94/10202.

Claim 34 has been amended to an isolated nucleic acid encoding a humanized variant of a parent anti-VEGF antibody which parent antibody comprises non-human variable domains, wherein said humanized variant (a) binds human VEGF with a K_d value of no more than about 1 x 10^{-8}M, said K_d value being no more than about 6-fold of the K_d value of said parent antibody; (b) has an ED50 value of no more than about 5nM for inhibiting VEGF-induced proliferation of endothelial cells *in vitro*; and (c) inhibits VEGF-induced angiogenesis *in vivo*, wherein 5mg/kg of said humanized variant inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model. Thus, an anti-VEGF antibody molecule encoded by the claimed nucleic acid must possess at least the following properties: 1) humanized; 2) strong binding affinity to human VEGF (i.e., K_d of no more than about 1 x 10^{-8}M), which is no more than about 6-fold of the parent anti-VEGF antibody; 3) ability to inhibit VEGF-induced endothelial cell proliferation *in vitro* at a low dose (an ED50 value of no more than about 5nM); and 4) ability to inhibit VEGF-induced angiogenesis *in vivo* with high efficacy (*i.e.*, 5mg/kg of the molecule inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model). Accordingly, to render the present claim obvious, it must be shown that one of ordinary

skill in the art would have been motivated and had a reasonable expectation of success to produce an isolated nucleic acid encoding a humanized anti-VEGF antibody variant having all of the above recited properties. Applicants contend to the contrary.

As disclosed in the specification of the present application, while non-human anti-VEGF neutralizing antibodies capable of suppressing angiogenesis related conditions (including the growth of a variety of human tumor cell lines in nude mice) were known in the art, the present invention is aimed at humanized anti-VEGF antibodies and anti-VEGF antibody variants with desirable properties from a therapeutic perspective. See specification at page 2, lines 19-29. The invention resulted from a series of experiments employing different approaches for humanizing an anti-VEGF antibody. For example, Example 1 of the application describes humanization of a murine anti-VEGF antibody A4.6.1 using the consensus sequences for the human heavy chain subgroup III and the light chain subgroup k I as the framework sequences. A series of humanized F(ab) fragments and IgG1 were generated. One of the humanized variants, F(ab)-12, exhibited a VEGF binding affinity of K_d 1.8x 10^{-9}M, which is within 2-fold that of a chimeric F(ab) used as the standard to represent the binding affinity of the parent murine antibody A4.6.1. See page 55, line 13 and Table 3 on page 57. F(ab)-12 was used to construct a full length mAb, rhuMAb VEGF, which exhibited similar VEGF binding affinity as that of F(ab)-12. In addition to having VEGF binding affinity comparable to counterpart murine anti-VEGF IgG1 (as shown in Table 4 at page 5), rhuMAb VEGF also showed biological activities that are essentially equivalent to the original murine anti-VEGF antibody A4.6.1. Pages 58-59 and Figures 3-4. Moreover, as discussed below, rhuMAb VEGF is currently being developed as an antiangiogenesis drug and used in several ongoing cancer clinical trials.

Example 2 describes humanization of a murine anti-VEGF antibody by randomizing a small set of framework residues and by monovalent display of the resultant library of antibody molecules on the surface of filamentous phage in order to identify high affinity framework sequences via affinity-based selection. Phage selected humanized variants can be subjected to additional mutagenesis for improved binding affinity. One of the selected clones, hu2.10V, exhibited a VEGF binding affinity of K_d

$9.3x10^{-9}M$, which is within 6-fold that of the A4.6.1 chimera standard. Table 7 on page 67.

Example 3 describes affinity enhancement of a humanized anti-VEGF antibody using CDR randomization, affinity maturation by monovalent Fab phage display, and cumulative combination of mutations. As a result, several antibody variants with high binding affinity were created, including Y0313-1, Y0238-3, and Y0317. See Table 15 on page 80 and discussion on page 81. Furthermore, several affinity-improved variants also effectively blocked VEGF-induced endothelial cell proliferation *in vitro*. Pages 79-80 and Figures 11-12.

Applicants do not dispute the notion that WO 94/10202 described monoclonal anti-VEGF antibodies having neutralizing and tumor inhibiting activities. Indeed, the murine anti-VEGF antibody A4.6.1 disclosed in WO 94/10202 was used to provide non-human CDRs for the humanized antibodies of the present invention. The cited reference also suggested in general producing humanized forms of murine antibodies. Applicants submit, however, that by simply applying conventional humanization methods to the murine antibodies of WO 94/10202, it would have been unexpected to produce a humanized anti-VEGF antibody variant which not only minimizes the HAMA immunogenicity of a parent murine antibody, but also 1) binds hVEGF with a strong affinity that is no more than about 6-fold of the parent antibody; 2) inhibits VEGF-induced endothelial cell proliferation *in vitro* at a low dose (an ED50 value of no more than about 5nM); and 3) inhibits VEGF-induced angiogenesis *in vivo* with a high efficacy (inhibits at least about 50% of tumor growth in an A673 *in vivo* tumor model at 5mg/kg).

The unexpected nature of the present invention is illustrated by the inventors' own initial work as described in the Examples of the application. Example 1 describes F(ab)-1, the first humanized variant which contains murine CDRs from A4.6.1 in a human framework and further contains a murine residue substitution at position H49 of the framework. See page 55, lines 13-16. As shown in Table 2 on page 56, binding of F(ab)-1 to VEGF decreased by over 1350-fold compared to that of the chimeric F(ab) standard. Such a humanized variant, while being less immunogenic in human, would nonetheless have no therapeutic efficacy because of its extremely poor binding affinity. Subsequent to F(ab)-1, many other humanized variants were also made, yet they did not have binding

affinities strong enough to be therapeutically useful (Table 2 on page 56). And it was only after a series of individual and combined residue substitutions in light of conformational analysis of antibody domains, the preferred humanized variant, F(ab)-12, was obtained. Example 2 also describes an initial humanized anti-VEGF antibody, hu2.0. See page 63, lines 10-18. Binding of hu2.0 to VEGF was so weak as to be undetectable. The K_d value of hu2.0 was estimated at greater than $7x10^{-6}$M, which is over 4000-fold weaker than that of the chimeric standard (Table 7 on page 66). Several other humanized variants subsequently made did not gain desirable binding affinities neither. Thus, while the skilled artisan might have been motivated to try to improve the binding affinity of a humanized antibody following conventional methods, it would have been unexpected to increase the binding affinity of $7x10^{-6}$M by at least 700-fold to the extent as presently claimed (i.e., a K_d of no more than about $1x10^{-8}$M, which is no more than about 6-fold reduction from that of the parent antibody), much less to obtain a humanized anti-VEGF antibody with all the distinctive properties as currently claimed.

In support of the patentability of the present invention, Applicants submit that humanized anti-VEGF antibody variants encoded by the nucleic acid of the present invention have exhibited great therapeutic potentials for treating cancers in both animal model studies and clinical trials in cancer patients, following the present invention. Indeed, Genentech's anti-VEGF monoclonal antibody, AVASTIN™ (which is the rhuMAb VEGF disclosed in the current application), is currently in clinical trials in combination with chemotherapy. Phase II and III trials are being conducted in patients with colorectal cancer that has metastasized to other parts of the body; and one phase III trial is being conducted in patients with advanced breast cancer. Further information regarding Genentech's AVASTIN™ clinical trials can be found online at www.gene.com/gene/science/pipeline/trials. Applicants further note that prior to the present invention, no other parties successfully obtained humanized anti-VEGF antibodies that are therapeutically useful for cancer therapies. In contrast, however, immediately after the publication of PCT counterpart of the present application (WO98/45331, published October 15, 1998), other parties filed applications claiming subject matters substantially similar to that of the present invention. See, for example, the PCT publication WO00/34337 by Toagosei Co., Ltd. and Protein Design Labs, Inc.

(claiming priority to U.S. Application Serial No. 09/209,990, filed December 10, 1998). Thus, the lack of success in the art prior to the present invention, and the following actions by others immediately after the present invention are further indicative of unobviousness of the present invention.

Applicants submit that the claims are now in condition for allowance. An early Notice to that effect is respectfully requested. In the event that the Examiner wishes to discuss any aspect of this response or of the application, he is invited to telephone the undersigned attorney at (650)225-8674. Applicants will be pleased to submit documents necessary to advance this application to issuance.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, Applicants petition for any required relief including extension of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to Deposit Account No. 07-0630. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,
GENENTECH, INC.

Date: September 7, 2001

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(a) binds human VEGF with a K_d value of no more than about 1×10^{-8} M, said K_d value being no more than about 6-fold of the K_d value of said parent antibody;

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